American Journal of Medical Genetics 62:160–163 (1996)

Mild Phenotype Associated With Inv Dup 8 (q21.2–q22.3) of Maternal Origin

Rossella Tupler, Emanuela Pagliano, Laura Barbierato, Giovanni Lanzi, Paola Maraschio, and Elisa Fazzi

Biologia Generale e Genetica Medica (R.T., L.B., P.M.), and Divisione di Neuropsichiatria Infantile, Clinica Neurologica (E.P., G.L., E.F.), Università di Pavia, Pavia, Italy

We report on a girl with a de novo inverted duplication of chromosome 8 (q21.2–q22.3) associated with a mild phenotype. We were able to establish the maternal origin of the rearranged chromosome. We discuss the correlation between genotype and phenotype on the basis of a review of the findings from individuals with partial dup(8q).
© 1996 Wiley-Liss, Inc.

KEY WORDS: trisomy 8q21.2–q22.3, maternal origin, umbilical hernia, high arched palate, micrognathia, flat nasal bridge, mild mental retardation

INTRODUCTION

Partial duplications of chromosome arm 8q have been reported in at least 80 patients [Schinzel, 1994] and appear to result in several syndromes all distinct from trisomy 8 mosaicism. Patients carrying partial duplications of 8q have a high incidence of skeletal anomalies, congenital heart defects, and consistent but variable degrees of mental retardation [Walker and Bocian, 1987]. A large group of these partial duplications involves the segment 8q22-qter. The only report of trisomy of the 8q21.2–q22 region is that described by Bowen et al. [1983] in a kindred in which an ins(10;8)(q21;q21.2–q22) chromosome rearrangement segregated in four generations.

CLINICAL REPORT

A.G. is a girl born July 27, 1991. Pregnancy and delivery were normal, except for a reduction in fetal activity in the last 4 weeks as reported by the mother. The parents were healthy and unrelated, both 28 years old at the time of conception. She is the second of a sibship of two. No previous pregnancy losses, stillbirths, or infant death were referred. At birth she presented a large umbilical hernia and some minor anomalies such as hypertelorism, flat nasal bridge, high arched palate, and micrognathia. A cavernous hemangioma at the left buttock and a cafè-au-lait spot on the proximal part of the left leg were visible. At birth her weight was 3100 g (25th–50th centile), her length was 51 cm (50th–75th centile), and her head circumference was 35.5 cm (50th centile).

A.G. was referred to the Division of Child Neuropsychiatry of the University of Pavia when 12 months old and has been followed up to her present age of 31 months (Fig. 1). At that time her height was 72.5 cm (25th centile), her weight was 9 kg (25th centile), and her head circumference was 46.5 cm (50th centile). Outer canthal distance was 9.5 cm (>97th centile).

Neurological examination showed psychomotor delay associated with generalized hypotonia. The child appeared to be aware of and interested in her surroundings, grasping and exploring objects. Language was limited to simple babbling. The Developmental Quotient, using the Griffiths Scales at the age of 12 and 31 months, respectively, documented mild mental impairment (General Intelligence Quotient: 77).

The main investigations carried out included muscle enzyme tests (GOT, GPT, and CK), thyroid screening (FT3, FT4, and TSH), electrocardiogram, renal and abdominal sonography, carpus X-ray, and visual and acoustic evoked potentials, all with normal results. Brain magnetic resonance imaging indicated slight delay in myelinization. No midline malformations were observed. EEG recording revealed recurrent brief paroxysms with diffuse and atypical spike wave appearance. Ophthalmological investigations documented normal ocular motility and no refractive abnormalities. Bilateral fundus exploration evidenced hyperpigmentation of the posterior pole.

Received for publication March 27, 1995; revision received October 3, 1995.

Address reprint requests to Dr. Rossella Tupler, Biologia Generale e Genetica Medica, CP 217, 27100 Pavia, Italy.

© 1996 Wiley-Liss, Inc.

Fig. 1. The patient at age of 31 months.

Muscle sonogram showed a slight and irregular increase in echogenicity of muscle tissue, the pathological significance of which is uncertain. Electromyography showed bilaterally, in the muscle examined, abnormal spontaneous activity (fibrillation); motor unit potentials (MUPs) were normal but with percentage increase in polyphasic forms. Nerve conduction studies were normal.

MATERIALS AND METHODS

Cytogenetic Studies

Cytogenetic studies were performed on metaphase chromosomes obtained by standard methods from phytohemagglutinin (PHA) stimulated whole blood cultures. Chromosome spreads were processed for GTG and QFQ banding. High-resolution banding was obtained according to the technique of Dutrillaux and Viegas-Pequignot [1981] with minor modifications (D-actinomycin was added at a final concentration of 5.5 ng/ml, 45 min before harvesting).

In Situ Hybridization

Fluorescent in situ hybridization (FISH) was carried out on mitotic preparations with a chromosome 8 biotinylated painting library (ONCOR). The probe was denatured at 70°C for 10 min and then incubated at 37°C for 1 hr to allow annealing of repetitive sequences. Chromosome slides were denatured at 70°C in 70% formamide, 2 × SSC for 2 min and alcohol dehydrated. Hybridization was carried out in a moist chamber for 16 hr at 42°C. Posthybridization washes were performed in 50% formamide, 1 × SSC at 42°C for 10 min, and twice in 2 × SSC at 42°C for 10 min.

Signal detection was achieved by treatment with three alternating layers of fluoresceinated avidin and biotinylated goat antiavidin (A-2011 and BA-0300 respectively. Vector Laboratories, Burlingame, CA). After the final avidin treatment, DAPI staining and counterstaining with 0.5 mg/ml propidium iodide in PBS were carried out.

(CA)n Dinucleotides Probes

(CA)n dinucleotides markers Mfd 8 (D8S84 locus, 8q13–q21.2), Mfd 18 (D8S85 locus, 8q22–qter), and MYC (c-myc locus, 8q24) were tested using the primer sequences published by Tomfohrde et al. [1992] (Mfd 8, Mfd 18) and Polymeropoulos et al. [1992] (MYC).

PCR was performed with the thermostable enzyme Taq polymerase (1 U/sample) (SuperTaq, HT Biotechnology LTD, Cambridge, England) and a programmable PCR apparatus (PTC 100, Programmable Thermal Controller, MJ Research, Inc.).

Target sequences were amplified in a 15 μl reaction mixture containing 100 ng of genomic DNA in 50 mM KCl, 50 mM Tris-HCl (pH 9.0), 7 mM $MgCl_2$, 5 pmol of each primer (Isogen, Bioscience, Atenlaboratorium, Amsterdam), 0.2 mM dNTPs, 0.2 mg/ml BSA, and 16 mM $(NH_4)_2SO_4$. About 50 μl of mineral oil was overlaid on the reaction mixture to prevent evaporation.

Amplification was for 27 cycles; each cycle consisted of 1 min denaturation at 94°C, 2 min annealing at 56°C, and 1 min extension at 72°C. The final extension step was prolonged for 6 min.

All (CA)n dinucleotides probes were labelled with ^{35}S dATP. The PCR products were resolved by electrophoresis on 6% denaturing polyacrylamide gels and were detected by 1–3 d autoradiography.

RESULTS

Chromosome analysis of the patient showed an abnormal chromosome 8. High resolution banding suggested an inverted duplication of the region 8q21.2–q22.3. The karyotype was interpreted as: 46,XX, inv dup 8 (q21.2–q22.3) (Fig. 2). Parents' karyotypes were normal. FISH analysis with specific chromosome 8 painting probe confirmed the derivation from chromosome 8 (Fig. 3).

(CA)n dinucleotides polymorphism analysis showed the presence of 3 alleles, 2 of them deriving from the 2 homologous maternal chromosome 8 at D8S85 (Mfd 18) locus. Two alleles, one maternal and one paternal, were present at D8S84 (Mfd 8) and MYC loci (Fig. 4). Comparing our results to the published chromosome 8 linkage map [Tomfohrde et al., 1992], loci D8S84, and MYC resulted proximal and distal, respectively, to the breakpoints.

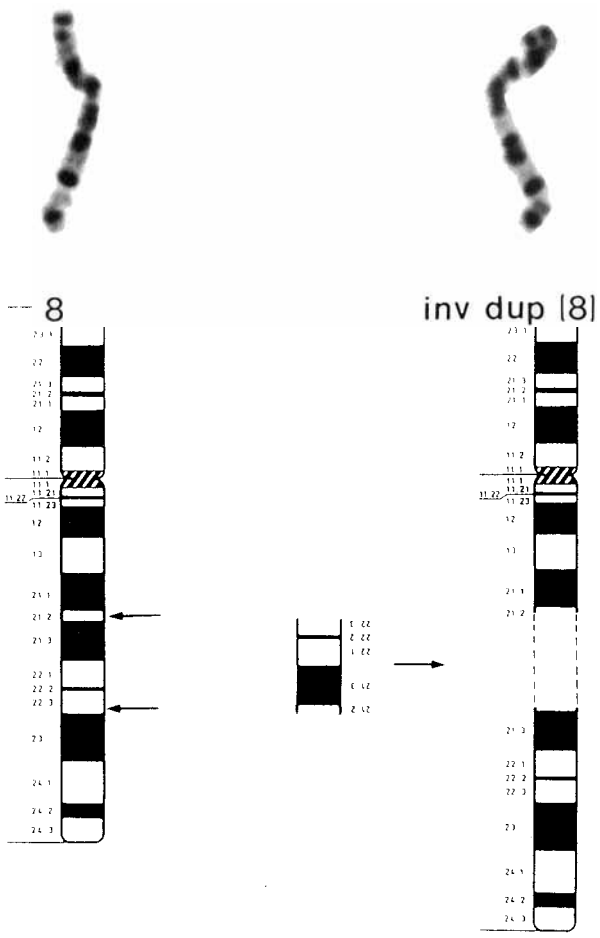

Fig. 2. The normal (left) and the abnormal chromosome 8 (right) with inverted duplication of the region q21.2–q22.3 (on the right) as illustrated in the idiogram below.

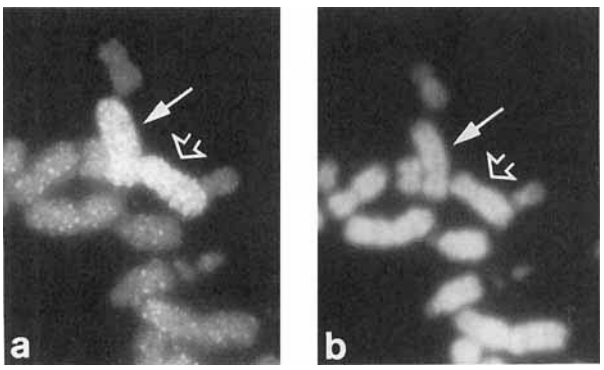

Fig. 3. **a:** Fluorescent in situ hybridization with a chromosome 8 painting library: open arrow points to the normal chromosome 8 and full arrow to the duplicated one. **b:** The same metaphase after DAPI staining.

DISCUSSION

Cytogenetic and molecular analysis of our patient showed a de novo inverted duplication of the region 8q21.2–q22.3. The presence of both maternal alleles at loci mapped in 8q21.2–q22 indicate an unequal inverted crossing over between homologous chromosomes during the maternal meiosis I as mechanism of formation of the abnormal chromosome.

Clinical evaluation revealed a mild mental retardation with slight neurological signs, associated with minor anomalies such as hypertelorism, flat bridge of nose, and high arched palate. She also had a cavernous hemangioma and a cafè-au-lait spot. Results of other investigations were normal. Her followup also showed normal growth and good improvement in her psychomotor development.

The duplication of the 8q21.3–q22.3 region is likely to be responsible for the abnormal phenotype of our patient. Nine patients carrying a duplication of the 8q21.2–q22 due to a familial chromosome insertion represent the sole description of individuals with a pure trisomy of that region [Bowen et al., 1983]. The major manifestations of these patients included mental retardation (present in all of them), highly arched or cleft palate (8/9), micrognathia (6/9), sloped shoulders (4–6/9), convulsions (4/9), camptodactyly (3/9), and pectus excavatum (2/9).

The phenotypic findings in our patient overlap with the more common ones, i.e., mild mental retardation, high arched palate, and micrognathia, found in most of the members of the family described by Bowen et al. [1983].

On the other hand, we stress the fact that the mechanism of origin of the abnormal chromosome of our patient is such to cause a maternal heterodisomy of the duplicated region that could be imprinted.

However, the duplication present in the family described by Bowen et al. [1983] is transmitted in both sexes through at least four generations without any differences in clinical expression regardless of the gender of the transmitting balanced carrier. This observation seems to exclude an imprinting effect in the region studied. Walker and Bocian [1987] failed to document a consistent pattern of malformations in their analysis of other different partial trisomies of the long arm of chromosome 8. Nevertheless they found a high incidence of cardiac malformations and skeletal anomalies, which are absent in the patients of Bowen et al. and in ours. This excludes the involvement of this region in the genesis of these defects.

ACKNOWLEDGMENTS

We are grateful to Dr. J. Opitz for his helpful suggestions on the clinical evaluation of our patient and Prof. M. Fraccaro for critically reading the manuscript. L. Barbierato was supported by Fondazione Anna Villa Rusconi.

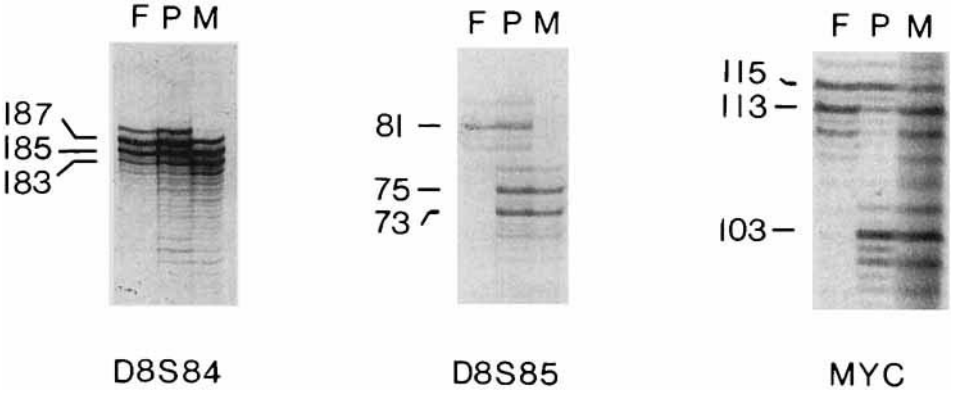

Fig. 4. Microsatellite polymorphism analyses of the patient (P) and her father (F) and mother (M) revealed the presence of three alleles at locus D8S85 in the patient and demonstrated the maternal origin of the abnormal chromosome 8. Loci D8S84 and MYC were not duplicated and are located, respectively, proximal and distal to the breakpoints. Numbers represent allele sizes expressed in basepair.

REFERENCES

Bowen P, Fitzgerald PH, Gardner RJM, Biederman B, Veale AMO (1983): Duplication 8q syndrome due to familial chromosome ins(10;8) (q21;q21.2 q22). Am J Med Genet 14:635–646.

Dutrillaux B, Viegas-Pequignot E (1981): High resolution R and G banding on the same preparation. Hum Genet 57:93–95.

Polymeropoulos MH, Xiao H, Merril CR (1992): Dinucleotide repeat polymorphism at the human c-myc oncogene locus (MYC). Hum Mol Genet 1:65.

Schinzel A (1994): Human Cytogenetics Database. In Baraitser M, Winter RM (eds): "Oxford Medical Database."

Tomfohrde J, Wood S, Schertzer M, Wagner MJ, Wells DE, Parrish J, Sadler LA, Blanton SH, Daiger SP, Wang Z, Wilkie P, Weber JL (1992): Human chromosome 8 linkage map based on short tandem repeat polymorphisms: Effect on genotyping errors. Genomics 14:144–152.

Walker AP, Bocian M (1987): Partial duplication 8q12-q21.2 in two sibs with maternally derived insertional and reciprocal translocations: Case reports and review of partial duplications of chromosome 8. Am J Med Genet 27:3–22.